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=> file medline hcaplus biosis biotechds scisearch embase
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=> (dityrosine or di-tyrosine or tyrosyl-tyrosyl) and crosslinking
(DITYROSINE IS NOT A RECOGNIZED COMMAND
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=> s (dityrosine or di-tyrosine or tyrosyl-tyrosyl) and crosslinking
L1          200 (DITYROSINE OR DI-TYROSINE OR TYROSYL-TYROSYL) AND CROSSLINKING

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2           131 DUP REM L1 (69 DUPLICATES REMOVED)

=> s l2 and lipase B
L3             0 L2 AND LIPASE B

=> s l2 and lipase
L4             2 L2 AND LIPASE

=> d l4 1-2 ibib ab

L4   ANSWER 1 OF 2  HCAPLUS  COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER:         2002:392180  HCAPLUS
DOCUMENT NUMBER:          136:382547
TITLE:                    Stabilization of proteins and enzymes by
                          tyrosyl-tyrosyl crosslinking
INVENTOR(S):              Marshall, Christopher P.; Hoffman, Alexander; Errico,
                          Joseph P.; Marshall, Paul B.
PATENT ASSIGNEE(S):       USA
SOURCE:                   U.S. Pat. Appl. Publ., 79 pp., Cont.-in-part of Appl.
                          No. PCT/US00/28595.
                          CODEN: USXXCO
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   2
PATENT INFORMATION:

     PATENT NO.        KIND  DATE           APPLICATION NO.   DATE
     ---------------   ----  --------       ---------------   --------
     US 2002061549      A1   20020523       US 2001-837235    20010418
     WO 2001029247      A1   20010426       WO 2000-US28595   20001016
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W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-159763P P 19991015
WO 2000-US28595 A2 20001016

AB The invention concerns methods for stabilizing polypeptides and polypeptide complexes, and the polypeptides and polypeptide complexes stabilized using the methods. To achieve stabilization, a cross-link reaction is controlled such that polypeptides and polypeptide complexes maintain their original functionality. In one embodiment, the invention provides a method for the identification of amino acid residues which, when cross-linked, are least disruptive to the structure and function of the polypeptide or polypeptide complex. In another embodiment, the invention provides a method for mutagenesis of identified residues to further control the cross-link reaction. Polypeptides and polypeptide complexes so stabilized can be utilized under a wide variety of physiol. and non-physiol. conditions. Further, the cross-link methodol. disclosed herein may preclude the need for addn. of exogenous structures to engineered proteins and complexes, such as peptide linkers that could be immunogenic and/or significantly decrease efficacy. In another embodiment, the invention provides a method for statistical anal. of databases of structural and/or sequence information available for polypeptides and polypeptide complexes to be stabilized. The statistical anal. identifies suitable residue pairs which are least likely to be disruptive of structure and function when cross-linked. Further, in a polypeptide chain or chains to be cross-linked, potentially undesirable reactive side-chains may be masked and protected, or altered using site-directed mutagenesis, e.g., to introduce a maximally conservative point mutation that will not support the cross-link reaction. The cross-link reaction conditions may also be adjusted to prevent undesired cross-links or other undesired side-effects. At residues identified as desirable positions for **crosslinking**, reactive side-chains may be introduced by site-directed mutagenesis, and the cross-link reaction is carried out using the conditions identified above.

L4 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2001:300896 HCAPLUS
DOCUMENT NUMBER: 134:323140
TITLE: Stabilization of proteins and enzymes by **tyrosyl-tyrosyl crosslinking**
INVENTOR(S): Marshall, Christopher P.; Hoffman, Alexander; Errico, Joseph P.; Marshall, Paul B.
PATENT ASSIGNEE(S): Avatar Medical, Llc, USA
SOURCE: PCT Int. Appl., 140 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001029247	A1	20010426	WO 2000-US28595	20001016

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1282722 A1 20030212 EP 2000-973574 20001016

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL

US 2002061549 A1 20020523 US 2001-837235 20010418

PRIORITY APPLN. INFO.: US 1999-159763P P 19991015

WO 2000-US28595 W 20001016

AB The invention described herein comprises methods for stabilizing polypeptides and polypeptide complexes by the introduction of intra-polypeptide and/or inter-polypeptide **tyrosyl-tyrosyl** bonds. The stabilization methods include controlled oxidative cross-link reaction such that polypeptides and polypeptide complexes maintain their original functionality. Embodiments of the invention outlining methods for identification of amino acid residues which when cross-linked are least disruptive to the structure and function of the polypeptides or polypeptide complex; as well as methods for mutagenesis for identifying residues to further control the cross-link reaction; and statistical anal. of the data base for the identification suitable residue pairs which are least likely to be disruptive of structure and function when cross-linked. Detailed cross-linked procedures and reaction conditions are exemplified and discussed.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s l2 and (protein or polypeptide) and dna
L5 3 L2 AND (PROTEIN OR POLYPEPTIDE) AND DNA

=> d l5 1-3 ibib ab

L5 ANSWER 1 OF 3 MEDLINE on STN

ACCESSION NUMBER: 95021520 MEDLINE

DOCUMENT NUMBER: PubMed ID: 7935621

TITLE: The role of **dityrosine** formation in the **crosslinking** of CUT-2, the product of a second cuticlin gene of Caenorhabditis elegans.

AUTHOR: Lassandro F; Sebastiano M; Zei F; Bazzicalupo P

CORPORATE SOURCE: International Institute of Genetics and Biophysics, Naples, Italy.

SOURCE: Molecular and biochemical parasitology, (1994 May) 65 (1) 147-59.
Journal code: 8006324. ISSN: 0166-6851.

PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-X74838

ENTRY MONTH: 199411

ENTRY DATE: Entered STN: 19941222
Last Updated on STN: 19941222
Entered Medline: 19941123

AB A second cuticlin gene, cut-2, of the nematode Caenorhabditis elegans, has been isolated and its genomic and cDNA sequences determined. The gene codes for a component of cuticlin, the insoluble residue of nematode cuticles. Conceptual translation of cut-2 reveals a 231-amino acid secreted **protein** which, like CUT-1, begins with a putative signal peptide of 16 residues. The central part of the **protein** consists of 13 repetitions of a short hydrophobic motif, which is often degenerated with substitutions and deletions. Parts of this motif are present also in CUT-1 (Caenorhabditis elegans) as well as in several **protein** components of the larval cuticle and of the eggshell layers of various insects (Locusta migratoria, Ceratitis capitata and Drosophila species). These sequence similarities are related to the

similar functions of these proteins: they are all components of extracellular insoluble protective layers. Immunolocalisation and transcription analysis suggest that CUT-2 contributes to the cuticles of all larval stages and that it is not stage-specific. Analysis by reverse transcriptase-PCR suggests that it is not stage-specific. Analysis by reverse transcriptase-PCR suggests that transcription is not continuous throughout larval development but occurs in peaks which precede the moults. **Dityrosine** has been detected in the cuticle of nematodes and of insects; formation of **dityrosine** bridges may be one of the cross-linking mechanisms contributing to the insolubility of cuticlins. Recombinant, soluble CUT-2 is shown to be an excellent substrate for an in vitro cross-linking reaction, catalysed by horseradish peroxidase in the presence of H2O2, which results in the formation of insoluble, high-molecular weight CUT-2 and of **dityrosine**.

L5 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1991:256135 HCAPLUS
DOCUMENT NUMBER: 114:256135
TITLE: Optical studies of molecular motions: using fluorescence anisotropy decays to determine the shapes of dye molecules, proteins, and nucleosomes
AUTHOR(S): Small, Enoch W.; Libertini, Louis J.; Brown, David W.; Small, Jeanne Rudzki
CORPORATE SOURCE: Dep. Biochem. Biophys., Oregon State Univ., Corvallis, OR, 97331-6503, USA
SOURCE: Optical Engineering (Bellingham, WA, United States) (1991), 30(3), 345-55
CODEN: OPEGAR; ISSN: 0091-3286
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Mols. undergo Brownian rotational diffusion, continually tumbling through soln. If a mol. contains a fluorescent chromophore with an appropriate excited-state lifetime, and a soln. of such mols. is excited with a brief flash of polarized laser light, then it is possible to measure the rates at which the mols. tumbles. One can measure the anisotropy of the decay of the fluorescence, a measure of the depolarization of the fluorescence in time. The rates of rotational diffusion are sensitive to the shape of the mol. The mol. shape information available from the anisotropy decay is examd. Three systems are described. Studies on the dye Rose Bengal (mol. wt. .apprx.1000) suggest that an approxn. used in rotational diffusion theory, that the solvent mols. are very small compared to the solute, is valid even for this relatively small mol. Next, the effects of calcium concn. on the rotational diffusion of the **protein** calmodulin (mol. wt. .apprx.17,000) derivatized by photoinduced **crosslinking** to form a **dityrosine** fluorophore were examd. In the presence of sufficiently high calcium ion concn., this crosslinked calmodulin shows a single exponential anisotropy decay consistent with the rotational diffusion of the extended dumbbell structure found for calmodulin by x-ray crystallog. At low calcium concns., the crosslinked calmodulin rotates considerably faster, suggesting a much more compact shape; also, this anisotropy decay shows short correlation times that are interpreted as arising from a segmental flexibility not evident for crosslinked calmodulin at high calcium concn. Finally, a transition is examd. that is obsd. at very low salt concns. for nucleosome core particles, relatively large complexes (mol. wt. .apprx.204,000) derived from the chromatin of higher organisms and comprised of eight histone mols. and 145 base pairs of **DNA** was examd. The anisotropy decay of ethidium bound to the **DNA** indicates that core particles exposed to low ionic strength are considerably elongated relative to the shape at higher ionic strength.

L5 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1988:566634 HCAPLUS
DOCUMENT NUMBER: 109:166634
TITLE: Molecular shapes from rotational diffusion: dye

molecules, proteins and nucleosomes
AUTHOR(S): Small, Enoch W.; Libertini, Louis J.; Rudzki Small, Jeanne
CORPORATE SOURCE: Dep. Biochem. Biophys., Oregon State Univ., Corvallis, OR, 97331, USA
SOURCE: Proceedings of SPIE-The International Society for Optical Engineering (1988), 909(Time-Resolved Laser Spectrosc. Biochem.), 97-107
CODEN: PSISDG; ISSN: 0277-786X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Shape information available from measurements of fluorescence anisotropy decays is examd. Anisotropy decays for 3 systems are reported and the kinds of information which may be obtained examd. Results for the dye Rose Bengal (.apprx.1000 daltons) suggest that an approxn. used in rotational diffusion theory, that the solvent mols. are very small compared to the solute, is valid even for this relatively small mol. Second, the effects of Ca concn. on rotational diffusion of the **protein** calmodulin (.apprx.17,000 daltons) derivatized by **crosslinking** to form a **dityrosine** fluorophore are examd. In the presence of sufficiently high Ca ion concn., this crosslinked calmodulin shows a single exponential anisotropy decay which indicates rotational diffusion consistent with the extended dumbbell structure found for calmodulin by x-ray crystallog. At low Ca concns., the crosslinked calmodulin rotates considerably faster, suggesting a much more compact shape; also, this anisotropy decay includes considerably shorter correlation times which are interpreted as arising from a segmental flexibility not evident for crosslinked calmodulin at high Ca concn. Finally, a transition which is obsd. at very low salt concns. of nucleosome core particles, relatively large complexes (.apprx.200,000 daltons) derived from chromatin and comprised of 8 histone mols. and 145 base pairs of **DNA**, is examd. The anisotropy decay of ethidium bound to the **DNA** indicates that core particles exposed to low ionic strength are considerably elongated relative to the shape at higher ionic strength.

```
=> s cross-linking tyrosine by oxidation
L6            0 CROSS-LINKING TYROSINE BY OXIDATION

=> s cross-linking tyrosine and (oxidation or oxidizing agent?)
L7            0 CROSS-LINKING TYROSINE AND (OXIDATION OR OXIDIZING AGENT?)

=> s tyrosine cross-link? and (oxidation or oxidizing agent?)
L8           36 TYROSINE CROSS-LINK? AND (OXIDATION OR OXIDIZING AGENT?)

=> dup rem l8
PROCESSING COMPLETED FOR L8
L9            17 DUP REM L8 (19 DUPLICATES REMOVED)

=> d l9 1-17 ibib ab

L9   ANSWER 1 OF 17     MEDLINE on STN                    DUPLICATE 1
ACCESSION NUMBER:   2004061812     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 14717612
TITLE:              Copper mediates dityrosine cross-linking of Alzheimer's
                    amyloid-beta.
AUTHOR:             Atwood Craig S; Perry George; Zeng Hong; Kato Yoji; Jones
                    Walton D; Ling Ke-Qing; Huang Xudong; Moir Robert D; Wang
                    Dandan; Sayre Lawrence M; Smith Mark A; Chen Shu G; Bush
                    Ashley I
CORPORATE SOURCE:   Institute of Pathology, Case Western Reserve University,
                    Cleveland, Ohio 44106, USA.. csa@medicine.wisc.edu
CONTRACT NUMBER:    AG19356 (NIA)
     P50AG08012 (NIA)
```

R01 AG14249 (NIA)
SOURCE: Biochemistry, (2004 Jan 20) 43 (2) 560-8.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200405
ENTRY DATE: Entered STN: 20040207
Last Updated on STN: 20040526
Entered Medline: 20040525

AB We have previously reported that amyloid Abeta, the major component of senile plaques in Alzheimer's disease (AD), binds Cu with high affinity via histidine and tyrosine residues [Atwood, C. S., et al. (1998) J. Biol. Chem. 273, 12817-12826; Atwood, C. S., et al. (2000) J. Neurochem. 75, 1219-1233] and produces H(2)O(2) by catalyzing the reduction of Cu(II) or Fe(III) [Huang, X., et al. (1999) Biochemistry 38, 7609-7616; Huang, X., et al. (1999) J. Biol. Chem. 274, 37111-37116]. Incubation with Cu induces the SDS-resistant oligomerization of Abeta [Atwood, C. S., et al. (2000) J. Neurochem. 75, 1219-1233], a feature characteristic of neurotoxic soluble Abeta extracted from the AD brain. Since residues coordinating Cu are most vulnerable to **oxidation**, we investigated whether modifications of these residues were responsible for Abeta cross-linking. SDS-resistant oligomerization of Abeta caused by incubation with Cu was found to induce a fluorescence signal characteristic of **tyrosine cross-linking**. Using ESI-MS and a dityrosine specific antibody, we confirmed that Cu(II) (at concentrations lower than that associated with amyloid plaques) induces the generation of dityrosine-cross-linked, SDS-resistant oligomers of human, but not rat, Abeta peptides. The addition of H2O2 strongly promoted Cu-induced dityrosine cross-linking of Abeta1-28, Abeta1-40, and Abeta1-42, suggesting that the oxidative coupling is initiated by interaction of H2O2 with a Cu(II) tyrosinate. The dityrosine modification is significant since it is highly resistant to proteolysis and is known to play a role in increasing structural strength. Given the elevated concentration of Cu in senile plaques, our results suggest that Cu interactions with Abeta could be responsible for causing the covalent cross-linking of Abeta in these structures.

L9 ANSWER 2 OF 17 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2003342869 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12857790
TITLE: Effects of oxidative and nitrative challenges on alpha-synuclein fibrillogenesis involve distinct mechanisms of protein modifications.
AUTHOR: Norris Erin H; Giasson Benoit I; Ischiropoulos Harry; Lee Virginia M-Y
CORPORATE SOURCE: Center for Neurodegenerative Disease Research and the Department of Pathology and Laboratory Medicine, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania 19104, USA.
CONTRACT NUMBER: AG 09215 (NIA)
SOURCE: Journal of biological chemistry, (2003 Jul 18) 278 (29) 27230-40.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200308
ENTRY DATE: Entered STN: 20030724
Last Updated on STN: 20030828
Entered Medline: 20030827

AB Filamentous inclusions of alpha-synuclein protein are hallmarks of neurodegenerative diseases collectively known as synucleinopathies.

Previous studies have shown that exposure to oxidative and nitrative species stabilizes alpha-synuclein filaments in vitro, and this stabilization may be due to dityrosine cross-linking. To test this hypothesis, we mutated tyrosine residues to phenylalanine and generated recombinant wild type and mutant alpha-synuclein proteins. alpha-Synuclein proteins lacking some or all tyrosine residues form fibrils to the same extent as the wild type protein. Tyrosine residues are not required for protein cross-linking or filament stabilization resulting from transition metal-mediated **oxidation**, because higher Mr SDS-resistant oligomers and filaments stable to chaotropic agents are detected using all Tyr --> Phe alpha-synuclein mutants. By contrast, cross-linking resulting from exposure to nitrating agents required the presence of one or more tyrosine residues. Furthermore, **tyrosine cross-linking** is involved in filament stabilization, because nitrating agent-exposed assembled wild type, but not mutant alpha-synuclein lacking all tyrosine residues, was stable to chaotropic treatment. In addition, the formation of stable alpha-synuclein inclusions in intact cells after exposure to oxidizing and nitrating species requires tyrosine residues. These findings demonstrate that nitrative and/or oxidative stress results in distinct mechanisms of alpha-synuclein protein modifications that can influence the formation of stable alpha-synuclein fibrils.

L9 ANSWER 3 OF 17 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2003185368 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12703970
TITLE: Dityrosine cross-linked Abeta peptides: fibrillar beta-structure in Abeta(1-40) is conducive to formation of dityrosine cross-links but a dityrosine cross-link in Abeta(8-14) does not induce beta-structure.
AUTHOR: Yoburn Joshua C; Tian Wenqiang; Brower Justin O; Nowick James S; Glabe Charles G; Van Vranken David L
CORPORATE SOURCE: Department of Chemistry, University of California, Irvine, California 92697, USA.
CONTRACT NUMBER: 5T32CA009054 (NCI)
GM-49076 (NIGMS)
GM-54523 (NIGMS)
SOURCE: Chemical research in toxicology, (2003 Apr) 16 (4) 531-5. Journal code: 8807448. ISSN: 0893-228X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200403
ENTRY DATE: Entered STN: 20030422
Last Updated on STN: 20040324
Entered Medline: 20040323

AB Recent reports by Galeazzi and co-workers demonstrated the susceptibility of Abeta(1-42) to undergo dityrosine formation via peroxidase-catalyzed **tyrosine cross-linking**. We have formed dityrosine cross-links in Abeta(1-40) using these enzymatic conditions as well as a copper-H(2)O(2) method. The efficiency of dityrosine cross-link formation is strongly influenced by the aggregation state of Abeta; more dityrosine is formed when copper-H(2)O(2) or horseradish peroxidase-catalyzed **oxidation** is applied to fibrillar Abeta vs soluble Abeta. Once formed, dityrosine cross-links are susceptible to further oxidative processes and it appears that cross-links formed in soluble Abeta react through these pathways more readily than those formed in fibrillar Abeta. Because preorganization of fibrils affects the efficiency of dityrosine formation, we examined the effect of dityrosine formation upon local peptide conformation by assessing the solution structure of a small dityrosine dimer derived from Abeta(8-14). Two-dimensional (1)H NMR studies of the short dityrosine dimer offer no evidence of structure. Thus, the fibrillar structure of Abeta enhances formation of dityrosine cross-links, but dityrosine cross-links do not seem to enhance local secondary structure.

L9 ANSWER 4 OF 17 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2003166899 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12684005
TITLE: A novel heme and peroxide-dependent tryptophan-**tyrosine cross-link** in a mutant of cytochrome c peroxidase.
AUTHOR: Bhaskar B; Immoos Chad E; Shimizu Hideaki; Sulc Filip; Farmer Patrick J; Poulos Thomas L
CORPORATE SOURCE: Department of Molecular Biology and Biochemistry, School of Biological Sciences, University of California, Irvine, CA 92697-3900, USA.
CONTRACT NUMBER: GM 42614 (NIGMS)
SOURCE: Journal of molecular biology, (2003 Apr 18) 328 (1) 157-66. Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1MK8; PDB-1MKQ; PDB-1MKR; PDB-1ML2
ENTRY MONTH: 200305
ENTRY DATE: Entered STN: 20030410
Last Updated on STN: 20030514
Entered Medline: 20030513

AB The crystal structure of a cytochrome c peroxidase mutant where the distal catalytic His52 is converted to Tyr reveals that the tyrosine side-chain forms a covalent bond with the indole ring nitrogen atom of Trp51. We hypothesize that this novel bond results from peroxide activation by the heme iron followed by **oxidation** of Trp51 and Tyr52. This hypothesis has been tested by incorporation of a redox-inactive Zn-protoporphyrin into the protein, and the resulting crystal structure shows the absence of a Trp51-Tyr52 cross-link. Instead, the Tyr52 side-chain orients away from the heme active-site pocket, which requires a substantial rearrangement of residues 72-80 and 134-144. Additional experiments where heme-containing crystals of the mutant were treated with peroxide support our hypothesis that this novel Trp-Tyr cross-link is a peroxide-dependent process mediated by the heme iron.

L9 ANSWER 5 OF 17 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2004:197086 BIOSIS
DOCUMENT NUMBER: PREV200400197645
TITLE: Substitution of tyrosine with cysteine in human alpha -synuclein promotes protein aggregation and neurotoxicity.
AUTHOR(S): Zhou, W. [Reprint Author]; Freed, C. R. [Reprint Author]
CORPORATE SOURCE: Div. Clin. Pharmacol., and the Neurosci. Program, Dept. of Med., Univ. of Colo. Hlth. Sci. Ctr., Denver, CO, USA
SOURCE: Society for Neuroscience Abstract Viewer and Itinerary Planner, (2003) Vol. 2003, pp. Abstract No. 298.13. http://sfn.scholarone.com. e-file.
Meeting Info.: 33rd Annual Meeting of the Society of Neuroscience. New Orleans, LA, USA. November 08-12, 2003. Society of Neuroscience.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 14 Apr 2004
Last Updated on STN: 14 Apr 2004

AB Accumulation of alpha-synuclein in Lewy bodies and Lewy neurites is a pathologic hallmark of Parkinson's disease. **Oxidation** and nitration of alpha-synuclein lead to the formation of stable dimers and oligomers through di-**tyrosine cross-linking**.
Oxidative dimer formation is the critical rate-limiting step for fibrillogenesis of alpha-synuclein. We have substituted cysteine (C) for tyrosine (Y) at positions 39, 125, 133, 136 in human wild-type

alpha-synuclein, and in A53T and A30P mutant alpha-synuclein. Results have shown that expression of Y39C or Y125C mutant alpha-synuclein, but not Y133C or Y136C, significantly increased cytoplasmic inclusions and cellular toxicity in a rat dopaminergic cell line (N27 cells) and in 293 cells. Under oxidizing conditions in vitro, recombinant Y39C or Y125C proteins showed more abundant dimer and polymer formation than wild type alpha-synuclein. Transgenic mice expressing Y39C human alpha-synuclein under control of the mouse Thy-1 promoter were generated. Neuronal accumulation of Y39C human alpha-synuclein was found in neocortex, hippocampus and brainstem. Transgenic animals had impaired motor performance in rotorod testing. We conclude that molecular substitutions which increase dimer formation at positions 39 and 125 can accelerate protein aggregation and neuronal toxicity of human alpha-synuclein.

L9 ANSWER 6 OF 17 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:325621 BIOSIS
DOCUMENT NUMBER: PREV200300325621
TITLE: STRUCTURAL MODIFICATIONS OF HUMAN alpha - SYNUCLEIN: EFFECTS ON PROTEIN AGGREGATION AND NEUROTOXICITY.
AUTHOR(S): Zhou, W. [Reprint Author]; Freed, C. R. [Reprint Author]
CORPORATE SOURCE: Div Clinical Pharmacology, the Neuroscience Program, Univ of Colorado Health Sci Ctr, Denver, CO, USA
SOURCE: Society for Neuroscience Abstract Viewer and Itinerary Planner, (2002) Vol. 2002, pp. Abstract No. 689.5. http://sfn.scholarone.com. cd-rom.
Meeting Info.: 32nd Annual Meeting of the Society for Neuroscience. Orlando, Florida, USA. November 02-07, 2002. Society for Neuroscience.
DOCUMENT TYPE: Conference; (Meeting)
Conference; (Meeting Poster)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 16 Jul 2003
Last Updated on STN: 16 Jul 2003

AB The deposition of alpha-synuclein and other cell proteins in Lewy bodies in midbrain dopamine neurons is a pathological hallmark of Parkinson's disease (PD). In vitro, **oxidation** and nitration of alpha-synuclein leads to the formation of dimers, polymers and fibrils through di-**tyrosine cross-linking**, suggesting that the cross-linking process can seed and initiate protein precipitation. To determine if enhanced dimer formation can accelerate protein aggregation and increase neuronal toxicity, we have substituted cysteine (C) for tyrosine (Y) at positions 39, 125, 133, 136 in human wild-type alpha-synuclein, and in A53T and A30P mutant alpha-synuclein. To reduce the likelihood of cross-linking, phenylalanine (F) was substituted for tyrosine at the same sites. We examined aggregate formation and neurotoxic effects of these constructs in a rat dopaminergic cell line (N27 cells) by transient transfection. Results showed that expression of Y39C or Y125C mutant proteins led to large intracellular inclusions. Both proteins produced more cell death compared to wild type human alpha-synuclein. Overexpression of Y133C, Y136C and all four Y to F mutations did not generate inclusions and were not more cytotoxic than wild type control. Under oxidizing conditions in vitro, recombinant Y39C or Y125C proteins showed more abundant dimer and polymer formation than wild type alpha-synuclein. We conclude that increased dimer formation can accelerate protein aggregation and neuronal toxicity of alpha-synuclein.

L9 ANSWER 7 OF 17 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-05391 BIOTECHDS
TITLE: Novel dual oxidases involved in generation of reactive oxygen intermediates and in preoxidative reactions affecting biological functions e.g. cell division and tissue fibrosis, useful for treating disease e.g. cancer;
involving vector plasmid pBluescript-mediated gene transfer for expression in host cell, for use in gene

therapy

AUTHOR: LAMBETH J D; LASSEGUE B P; GRIENDLING K K; ARNOLD R S; CHENG G; SHARLING L; BENIAN G; EDENS W A

PATENT ASSIGNEE: UNIV EMORY

PATENT INFO: WO 2001087957 22 Nov 2001
APPLICATION INFO: WO 2000-US15573 15 May 2000
PRIORITY INFO: US 2000-222421 1 Aug 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-114232 [15]

AB DERWENT ABSTRACT:

NOVELTY - A protein (I) of dual oxidase (duox) capable of stimulating superoxide production or generating peroxidative reactions comprising a sequence (S1) of 1548, 1497, 593 or 590 amino acid sequences as given in the specification, or its fragments, or a conservative substitution, capable of generating reactive oxygen intermediates or generating peroxidative reactions, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a nucleotide sequence (II) encoding (I) , its fragment or their conservative substitution; (2) a vector (III) comprising (II); (3) a cell (IV) containing (III); (4) an antibody (V) generated against (I); (5) determining (M1) activity of a drug or a chemical, comprising (a) measuring enzymatic activity (I) to stimulate superoxide production or generate peroxidative reactions following administration of the drug or the chemical; (b) measuring binding of the drug or the chemical to (I); or (c) measuring the activity of the drug or chemical to modulate proliferative activity or peroxidative activity of (I); and (6) affecting (M2) cuticle biogenesis comprising administration to an organism with a cuticle of a composition which affects an activity of an protein comprising duox, of its fragment, or their conservative substitutions, where the activity is cuticle biogenesis.

WIDER DISCLOSURE - Disclosed as new are the following: (A) probes useful for the detection, localization and measurement of (II); (B) kits useful for detecting, measuring and locating (II) and (I) that are involved in reactive oxygen intermediates production; and (C) high throughput screening of drugs and chemicals which modulate the proliferative activity of (I) thereby affecting cell division, metabolic activity, cuticle formation, fibrosis and other biological functions involving oxidative reactions.

BIOTECHNOLOGY - Preferred Method: In (M1), the enzymatic activity is NADPH-dependent or NADPH-dependent superoxide generation, tetramethybenzidine **oxidation** or **tyrosine cross-linking**, where the enzymatic activity is assessed using intact cells, transfected cells, or their cell lysates.

ACTIVITY - Cytostatic: Hypotensive; Antiarteriosclerotic; Antipsoriatic; Cardiant; Antiparasitic; Vasotropic. No supporting data is given.

MECHANISM OF ACTION - Mitogenic and endocrine regulators; Gene therapy; Modulator of activity of expression of (I). No biological data provided.

USE - (I) or (III) is useful for stimulating superoxide formation or generating peroxidative reactions in vitro or in vivo. (I) is useful for evaluating a biological activity of a drug or a chemical, where the biological activity is preferably cuticle biogenesis, thyroid hormone biosynthesis or fibrosis especially lung fibrosis (claimed). (M1) is useful for determining activity of a drug or a chemical (claimed), useful as treatments for cancer, prostatic hypertrophy, benign prostatic hypertrophy, hypertension metabolic disease, fibrosis, atherosclerosis and many other disorders involving abnormal cell growth or proliferation, and a variety of parasitic diseases in both animals and crops. The method may also be used for the development of drugs or other therapies for treatment of conditions associated with abnormal growth which include the cancer, fibrosis, lung fibrosis, metabolic imbalances, thyroid imbalances, hyperthyroidism, psoriasis, prostatic hypertrophy, benign prostatic hypertrophy, cardiovascular disease, proliferation of vessels.

(M2) is useful for affecting cuticle biogenesis (claimed). (I) or mitogenic regulators, that catalyze thyroid hormone biosynthesis and in nematodes catalyze the biosynthesis of cuticle. (V) is useful as research and diagnostic reagents. (IV) is useful for stimulating tumor formation.

ADMINISTRATION - (I) is administered through oral including buccal and sublingual, rectal, parenteral, aerosol, nasal, intramuscular, subcutaneous, intradermal or topical route. Dosages is 0.1 mug-1 mg (preferably 25-500 mug).

EXAMPLE - A 535-base portion of an expressed sequence tag (EST zc92h03.rl; Genbank accession no. W52750) from human pancreatic islet was identified using the amino acid sequence of human gp91 phox as a query in a Blast search. The bacterial strain 595758 containing the EST sequence zc92h03.rl in the pBluescript SK-vector was purchased from ATCC. The DNA was sequenced using primers to T7 and T3 vector promoter as well as sequence-specific internal primers. The EST encoded a 440 amino acid partial cDNA exhibiting 24.4% identity to gp91 phox, including a stop codon corresponding to the C-terminus of gp91phox. 5'- and 3'- RACE (rapid amplification of cDNA ends) were carried out using human adult pancreas mRNA with the 5' RACE kit for Rapid Amplification of cDNA ends version 2.0. Polymerase chain reaction (PCR) was done with specific primers: 5'-RACE: primer 1,5'-GAAGTGGTGGGAGGCGAAGACATA-3'; primer 2, 5'-CCTGTCATACCTGGGACGGTCTGVG-3'; primer 3, 5'-GAGCACAGTGAGATGCCTGTTCAG-3'; primer 4, 5'-GGAAGGCAGCAGAGAGCAATGATG-3'; primer5, 5'-AGGTGGGATGCGGATGTTGAG-3' (for nested PCR); 3'-RACE primer 6, 5'-ACATCTGCGAGCGGCACTTCCAGA-3'; primer 7, 5'-AGCTCGTCAACAGGCAGGACCGAGC-3'; primer 8, 5'-TCTCCATCAGAATCCACCTTAGGC-3' (for nested PCR). To complete the sequence 5'-RACE was carried out using human thyroid Marathon-ready cDNA with primer 3 and adapter primer AP1, and primer 5 and adapter primer AP2. These procedures resulted in an additional 3.7 kb 5' region and a 1.5 kb 3' region. The cDNA for h-Duox2 showed a 4647 base pair open reading frame that was predicted to encode a protein of 1548 amino acids (175 kDa), and contained a consensus Kozak sequence, GGCATGC, at the translation start codon. The Duox2 cDNA sequence was found to be a larger form of a gp91phox homolog previously identified as an NADPH-oxidase in thyroid and termed p138Tox; the latter sequence did not contain the a peroxidase homology domain. h-Duox1 and h-Duox2 were 77% identical at the amino acid level. (86 pages)

L9 ANSWER 8 OF 17 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2001:710187 SCISEARCH
THE GENUINE ARTICLE: 467CX
TITLE: **Tyrosine cross-linking** of extracellular matrix is catalyzed by Duox, a multidomain oxidase/peroxidase with homology to the phagocyte oxidase subunit gp91 phox
AUTHOR: Edens W A; Sharling L; Cheng G J; Shapira R; Kinkade J M; Lee T; Edens H A; Tang X X; Sullards C; Flaherty D B; Benian G M; Lambeth J D (Reprint)
CORPORATE SOURCE: Emory Univ, Sch Med, Dept Biochem, Atlanta, GA 30322 USA (Reprint); Emory Univ, Sch Med, Dept Pathol, Atlanta, GA 30322 USA
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF CELL BIOLOGY, (20 AUG 2001) Vol. 154, No. 4, pp. 879-891.
Publisher: ROCKEFELLER UNIV PRESS, 1114 FIRST AVE, 4TH FL, NEW YORK, NY 10021 USA.
ISSN: 0021-9525.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 66

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB High molecular weight homologues of gp91 phox, the superoxide-generating subunit of phagocyte nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase, have been identified in human (h) and Caenorhahditis elegans (Ce), and are termed Duox for "dual oxidase"

because they have both a peroxidase homology domain and a gp91 phox domain. A topology model predicts that the enzyme will utilize cytosolic NADPH to generate reactive oxygen, but the function of the ecto peroxidase domain was unknown. Ce-Duox1 is expressed in hypodermal cells underlying the cuticle of larval animals. To investigate function, RNA interference (RNAi) was carried out in C. elegans. RNAi animals showed complex phenotypes similar to those described previously in mutations in collagen biosynthesis that are known to affect the cuticle, an extracellular matrix. Electron micrographs showed gross abnormalities in the cuticle of RNAi animals. In cuticle, collagen and other proteins are cross-linked via di- and trityrosine linkages, and these linkages were absent in RNAi animals. The expressed peroxidase domains of both Ce-Duox1 and h-Duox showed peroxidase activity and catalyzed crosslinking of free tyrosine ethyl ester. Thus, Ce-Duox catalyzes the cross-linking of tyrosine residues involved in the stabilization of cuticular extracellular matrix.

L9 ANSWER 9 OF 17 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 97239248 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9084910
TITLE: Nickel-dependent oxidative cross-linking of a protein.
AUTHOR: Gill G; Richter-Rusli A A; Ghosh M; Burrows C J; Rokita S E
CORPORATE SOURCE: Department of Chemistry, State University of New York at Stony Brook, New York 11794, USA.
SOURCE: Chemical research in toxicology, (1997 Mar) 10 (3) 302-9. Journal code: 8807448. ISSN: 0893-228X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199706
ENTRY DATE: Entered STN: 19970612
Last Updated on STN: 19970612
Entered Medline: 19970604

AB A model protein, ribonuclease A (bovine pancreas), was examined for its ability to coordinate Ni2+ and promote selective **oxidation**. In the presence of a peracid such as monopersulfate, HSO5-, nickel induced the monomeric RNase A to form dimers, trimers, tetramers, and higher oligomers without producing fragmentation of the polypeptide backbone. Co2+ and to a lesser extent Cu2+ exhibited similar activity. The nickel-dependent reaction appeared to result from a specific association between the protein and Ni2+ that allowed for transient and in situ **oxidation** of the bound nickel to yield intermolecular tyrosine-**tyrosine cross-links**. Macrocylic nickel complexes that had previously been shown to promote guanine **oxidation** were unable to mimic the activity of the free metal salt. Amino acid analysis of the protein dimer confirmed the expected consumption of one tyrosine per polypeptide and formation of dityrosine. The presence of excess tyrosine efficiently inhibited formation of the protein dimer and produced instead a ribonuclease-**tyrosine cross-link**. In contrast, high concentrations of the hydroxyl radical quenching agent mannitol only partially inhibited ribonuclease dimerization. The polypeptide-mediated activation of nickel and its subsequent reactivity mimic a process that could contribute to the adverse effects of nickel in vivo.

L9 ANSWER 10 OF 17 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1996:414336 HCAPLUS
TITLE: Nickel-dependent **oxidation** of a protein.
AUTHOR(S): Rokita, Steven E.; Gill, Gurpreet; Richter-Rusli, Angelika; Burrows, Cynthia J.
CORPORATE SOURCE: Department Chemistry and Biochemistry, University Maryland, College Park, MD, 20742, USA
SOURCE: Book of Abstracts, 212th ACS National Meeting, Orlando, FL, August 25-29 (1996), INOR-184. American Chemical Society: Washington, D. C.

CODEN: 63BFAF
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English

AB A model protein, RNase A, was characterized for its ability to coordinate and promote the oxidn.-redn. activity of Ni2+ for biopolymer modification. Flexible loop and coil regions of RNase were the expected targets of this oxidn. since they form relatively open conformations that may provide the necessary ligand field environment for reaction. Both scission of the peptide backbone and coupling of the side chains were possible. For RNase A, only covalent oligomerization was detected after addn. of a nickel salt and a peracid such as monopersulfate. Quenching studies and amino acid anal. suggest an intermediate tyrosyl radical was selectively generated to form interstrand tyrosine-**tyrosine cross-links**. Macrocyclic nickel complexes that were capable of mediating DNA oxidn. were not able to mimic the action of free nickel salts under these conditions. Furthermore, only cobalt and, to a much lesser extent, copper were found to induce an equiv. reaction among the numerous transition metals examd.

L9 ANSWER 11 OF 17 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 6

ACCESSION NUMBER: 1991:315810 BIOSIS
DOCUMENT NUMBER: PREV199192026325; BA92:26325
TITLE: CHEMICAL NATURE OF DNA PROTEIN CROSS-LINKS PRODUCED IN MAMMALIAN CHROMATIN BY HYDROGEN PEROXIDE IN THE PRESENCE OF IRON OR COPPER IONS.
AUTHOR(S): NACKERDIEN Z [Reprint author]; RAO G; CACCIUTTOLO M A; GAJEWSKI E; DIZDAROGLU M
CORPORATE SOURCE: CHEMICAL SCIENCE TECHNOLOGY LABORATORY, NATIONAL INSTITUTE STANDARDS TECHNOLOGY, GAITHERSBURG, MD 20899, USA
SOURCE: Biochemistry, (1991) Vol. 30, No. 20, pp. 4873-4879. CODEN: BICHAW. ISSN: 0006-2960.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 15 Jul 1991
Last Updated on STN: 16 Jul 1991

AB We report on the elucidation of DNA-protein cross-links formed in isolated mammalian chromatin upon treatment with H2O2 in the presence of iron or copper ions. Analysis of chromatin samples by gas chromatography/mass spectrometry after hydrolysis and derivatization showed the presence of 3-[(1,3-dihydro-2,4-dioxopyrimidin-5-yl)methyl]-L-tyrosine (thymne-**tyrosine cross-link**) on the basis of the gas chromatographic and mass spectrometric characteristics of the trimethylsilylated authentic compound. Other DNA-protein cross-links involving thymine and the aliphatic amino acids and cytosine and tyrosine, which were known to occur in nucleohistone .gamma.-irradiated under anoxic conditions, were not observed. This was due to inhibition by oxygen as clearly shown by experiments that were carried out using ionizing raiation under both oxic and anoxic conditions instead of using H2O2 and metal ions. However, oxygen did not inhibit formation of the thymine-**tyrosine cross-link** in .gamma.-irradiatd chromatin or in chromatin treated with H2O2 and metal ions. The yield of the thymine-**tyrosine cross-link** was higher upon treatment with H2O2/chelated Fe3+ ions than with H2O2/unchelated Fe3+ ions. By contrast, H2O/unchelated Cu2+ ions produced a higher yield than H2O2/chelated Cu2+ ions. Almost complete inhibition of cross-link formation was provided by the hydroxyl radical scavenger mannitol and dimethyl sulfoxide when H2O2/chelated metal ions were used. On the other hand scavengers only partially inhibited formation of cross-links when H2O2/unchelated metal ions were used, possibly indicating the site-specific nature of cross-linking. Superoxide dismutase afforded partial inhibition only when chelated ions were used. The mechanism underlying formation of this DNA-protein cross-link is thought to involve addition of the hydroxyl radical generated allyl radical of thymine to

carbon-3 of tyrosine followed by subsequent **oxidation** of the adduct radical.

L9 ANSWER 12 OF 17 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 91:295469 SCISEARCH
THE GENUINE ARTICLE: FM712
TITLE: CHEMICAL NATURE OF DNA-PROTEIN CROSS-LINKS PRODUCED IN MAMMALIAN CHROMATIN BY HYDROGEN-PEROXIDE IN THE PRESENCE OF IRON OR COPPER IONS
AUTHOR: NACKERDIEN Z; RAO G; CACCIUTTOLO M A; GAJEWSKI E; DIZDAROGLU M (Reprint)
CORPORATE SOURCE: NATL INST STAND & TECHNOL, CHEM SCI & TECHNOL LAB, GAITHERSBURG, MD, 20899; UNIV STELLENBOSCH, DEPT RADIOTHERAPY, TYGERBERG 7505, SOUTH AFRICA; UNIV MARYLAND, CATONSVILLE, MD, 21228; MARYLAND BIOTECHNOL INST, CTR MED BIOTECHNOL, BALTIMORE, MD, 21228
COUNTRY OF AUTHOR: USA; SOUTH AFRICA
SOURCE: BIOCHEMISTRY, (1991) Vol. 30, No. 20, pp. 4873-4879.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 51

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We report on the elucidation of DNA-protein cross-links formed in isolated mammalian chromatin upon treatment with H2O2 in the presence of iron or copper ions. Analysis of chromatin samples by gas chromatography/mass spectrometry after hydrolysis and derivatization showed the presence of 3-[(1,3-dihydro-2,4-dioxopyrimidin-5-yl) methyl]-L-tyrosine (thymine-**tyrosine cross-link**) on the basis of the gas chromatographic and mass spectrometric characteristics of the trimethylsilylated authentic compound. Other DNA-protein cross-links involving thymine and the aliphatic amino acids and cytosine and tyrosine, which were known to occur in nucleohistone gamma-irradiated under anoxic conditions, were not observed. This was due to inhibition by oxygen as clearly shown by experiments that were carried out using ionizing radiation under both oxic and anoxic conditions instead of using H2O2 and metal ions. However, oxygen did not inhibit formation of the thymine-**tyrosine cross-link** in gamma-irradiated chromatin or in chromatin treated with H2O2 and metal ions. The yield of the thymine-**tyrosine cross-link** was higher upon treatment with H2O2/chelated Fe3+ ions than with H2O2/unchelated Fe3+ ions. By contrast, H2O2/unchelated Cu2+ ions produced a higher yield than H2O2/chelated Cu2+ ions. Almost complete inhibition of cross-link formation was provided by the hydroxyl radical scavengers mannitol and dimethyl sulfoxide when H2O2/chelated metal ions were used. On the other hand, scavengers only partially inhibited formation of cross-links when H2O2/unchelated metal ions were used, possibly indicating the site-specific nature of cross-linking. Superoxide dismutase afforded partial inhibition only when chelated ions were used. The mechanism underlying formation of this DNA-protein cross-link is thought to involve addition of the hydroxyl radical generated allyl radical of thymine to carbon-3 of tyrosine followed by subsequent **oxidation** of the adduct radical.

L9 ANSWER 13 OF 17 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 89302970 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2545260
TITLE: Structure of a hydroxyl radical induced DNA-protein cross-link involving thymine and tyrosine in nucleohistone.
AUTHOR: Dizdaroglu M; Gajewski E; Reddy P; Margolis S A
CORPORATE SOURCE: Center for Chemical Technology, National Institute of Standards and Technology, Gaithersburg, Maryland 20899.
SOURCE: Biochemistry, (1989 Apr 18) 28 (8) 3625-8.
Journal code: 0370623. ISSN: 0006-2960.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198908
ENTRY DATE: Entered STN: 19900309
Last Updated on STN: 19900309
Entered Medline: 19890817

AB Hydroxyl radical induced formation of a DNA-protein cross-link involving thymine and tyrosine in nucleohistone is described. Hydroxyl radicals were generated in N2O-saturated aqueous solution by ionizing radiation. Samples of nucleohistone were hydrolyzed with HCl and trimethylsilylated. Analysis of irradiated samples by gas chromatography-mass spectrometry with selected-ion monitoring showed the presence of a thymine-**tyrosine cross-link** on the basis of typical fragment ions from the previously known mass spectrum of its trimethylsilyl derivative. The yield of this DNA-protein cross-link in nucleohistone was measured at incrementing doses of radiation and found to be a linear function of radiation dose between 14 and 300 Gy (J.kg-1). This yield amounted to 0.003 mumol.J-1. The mechanism of formation of this DNA-protein cross-link is thought to result from H atom abstraction by hydroxyl radicals from the methyl group of thymine followed by the addition of the resultant thymine radical to the carbon 3 position of the tyrosine ring and subsequent **oxidation** of the adduct radical.

L9 ANSWER 14 OF 17 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1984:37444 BIOSIS
DOCUMENT NUMBER: PREV198426037444; BR26:37444
TITLE: TYROSINE-**TYROSINE CROSS LINKS** IN IMMUNO GLOBULIN G GENERATION BY PER **OXIDATION** AND OCCURRENCE IN RHEUMATOID ARTHRITIS SYNOVIAL FLUIDS.
AUTHOR(S): MULLINAX F [Reprint author]; MULLINAX G L
CORPORATE SOURCE: MED COLL VA, RICHMOND, VA 23298, USA
SOURCE: Arthritis and Rheumatism, (1983) Vol. 26, No. 4 SUPPL, pp. S16.
Meeting Info.: 47TH ANNUAL MEETING OF THE AMERICAN RHEUMATISM ASSOCIATION, SAN ANTONIO, TEX., USA, JUNE 1-4, 1983. ARTHRITIS RHEUM.
CODEN: ARHEAW. ISSN: 0004-3591.
DOCUMENT TYPE: Conference; (Meeting)
FILE SEGMENT: BR
LANGUAGE: ENGLISH

L9 ANSWER 15 OF 17 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1982:300038 BIOSIS
DOCUMENT NUMBER: PREV198274072518; BA74:72518
TITLE: OZONE INDUCED FORMATION OF O O' DI **TYROSINE CROSS LINKS** IN PROTEINS.
AUTHOR(S): VERWEIJ H [Reprint author]; CHRISTIANSE K; VAN STEVENINCK J
CORPORATE SOURCE: SYLVIUS LAB, DEP MED BIOCHEMISTRY, WASSENAARSEWEG 72, 2333 AL LEIDEN
SOURCE: Biochimica et Biophysica Acta, (1982) Vol. 701, No. 2, pp. 180-184.
CODEN: BBACAQ. ISSN: 0006-3002.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH

AB Treatment of human blood spectrin, insulin, glucagon and ribonuclease with O3 resulted in covalent cross-linking of these proteins. This cross-linking was not reversed by treatment with dithiothreitol and could not be ascribed to -S-S bond formation. A concomitant O,O'-dityrosine formation was observed by spectrofluorometric analysis of the protein and by amino acid analysis and TLC of hydrolyzed protein samples. The protein cross-linking should be attributed to interpeptide O,O'-dityrosine bonds. **Oxidation** of proteins with horseradish peroxidase and H2O2 also

led to O,O'-dityrosine formation. Peroxidase-induced O,O'-dityrosine formation in galactose oxidase (D-galactose:oxygen 6-oxidoreductase, EC 1.1.3.9) caused a strong increase of enzyme activity. O3 treatment of galactose oxidase also led to O,O'-dityrosine formation with a concomitant 8-fold increase of enzyme activity.

L9 ANSWER 16 OF 17 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1978:190539 BIOSIS
DOCUMENT NUMBER: PREV197866003036; BA66:3036
TITLE: RELEASE OF OVO PEROXIDASE FROM SEA-URCHIN EGGS HARDENS THE FERTILIZATION MEMBRANE WITH **TYROSINE CROSS LINKS**.
AUTHOR(S): FOERDER C A [Reprint author]; SHAPIRO B M
CORPORATE SOURCE: DEP BIOCHEM, UNIV WASH, SEATTLE, WASH 98195, USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1977) Vol. 74, No. 10, pp. 4214-4218.
CODEN: PNASA6. ISSN: 0027-8424.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
AB One feature of fertilization is the alteration of the vitelline layer, by components released from the egg, to produce an elevated, covalently crosslinked, hard, insoluble, fertilization membrane. Evidence indicates that crosslinking and hardening [in Strongylocentrotus purpuratus] are caused by the production of di- and trityrosyl residues, by **oxidation** of protein-bound tyrosyl residues in the presence of peroxidase. Hardening of the fertilization membrane, as evidenced by its loss of solubility in 50 mM dithiothreitol, is inhibited by compounds known to inhibit many peroxidases. A peroxidase termed ovoperoxidase is released from eggs at fertilization. This enzyme is inhibited by the same compounds that inhibit hardening and at similar concentrations. Inhibitors of the ovoperoxidase and the hardening reaction include KCN, 3-amino-1,2,4-triazole, NaN3, phenylhydrazine, K4Fe(CN)6, sodium sulfite and glycine ethyl ester. Tyramine and N-acetyltyrosine both inhibit hardening but O-methyltyrosine does not. Di- and trityrosyl residues are found in acid hydrolysates of isolated, hardened fertilization membranes. These residues were identified by cellulose phosphate column chromatography, TLC and amino acid analysis. The amino acid data were used to estimate that there is 1 dityrosine crosslink/55,000 daltons of protein. By catalyzing the crosslinking of tyrosyl residues, the ovoperoxidase leads to the production of a hard fertilization membrane that blocks entry of additional sperm. Because peroxidases are spermicidal, a secondary function of the enzyme could be to kill sperm in the vicinity of the fertilized egg.

L9 ANSWER 17 OF 17 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1976:228598 BIOSIS
DOCUMENT NUMBER: PREV197662058598; BA62:58598
TITLE: FORMATION OF DI **TYROSINE CROSS LINKS** IN PROTEINS BY **OXIDATION** OF TYROSINE RESIDUES.
AUTHOR(S): AESCHBACH R; AMADO R; NEUKOM H
SOURCE: Biochimica et Biophysica Acta, (1976) Vol. 439, No. 2, pp. 292-301.
CODEN: BBACAQ. ISSN: 0006-3002.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: Unavailable
AB Enzymic **oxidation** of proteins with horseradish peroxidase (EC 1.11.1.7) and H2O2 at a basic pH value leads to an oxidative phenolic coupling of adjacent tyrosine residues forming cross-linked proteins. Dityrosine (3,3'-bityrosine) was identified as the cross-link in oxidized proteins by TLC, amino acid analysis and fluorescence measurements. Gel filtration experiments with oxidized insulin showed that the cross-linkage

is predominantly intermolecular. In tetranitromethane treated proteins, dityrosine could be identified after hydrolysis.

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=> d his

     (FILE 'HOME' ENTERED AT 17:24:19 ON 23 JUN 2004)

     FILE 'MEDLINE, HCAPLUS, BIOSIS, BIOTECHDS, SCISEARCH, EMBASE' ENTERED AT
     17:25:09 ON 23 JUN 2004
L1          200 S (DITYROSINE OR DI-TYROSINE OR TYROSYL-TYROSYL) AND CROSSLINKI
L2          131 DUP REM L1 (69 DUPLICATES REMOVED)
L3            0 S L2 AND LIPASE B
L4            2 S L2 AND LIPASE
L5            3 S L2 AND (PROTEIN OR POLYPEPTIDE) AND DNA
L6            0 S CROSS-LINKING TYROSINE BY OXIDATION
L7            0 S CROSS-LINKING TYROSINE AND (OXIDATION OR OXIDIZING AGENT?)
L8           36 S TYROSINE CROSS-LINK? AND (OXIDATION OR OXIDIZING AGENT?)
L9           17 DUP REM L8 (19 DUPLICATES REMOVED)

=> log y
COST IN U.S. DOLLARS                            SINCE FILE      TOTAL
                                                  ENTRY    SESSION
FULL ESTIMATED COST                               60.40      60.82

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)      SINCE FILE      TOTAL
                                                  ENTRY    SESSION
CA SUBSCRIBER PRICE                               -3.47      -3.47

STN INTERNATIONAL LOGOFF AT 17:33:45 ON 23 JUN 2004
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